

CLAIMS

1. A method for cleaving a protein or peptide at a specific site, c h a r a c t e r i z e d in that the method comprises the steps:

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- constructing at a predetermined cleavage site of the protein or peptide an amino acid sequence comprising at least two amino acids selected from the group comprising histidine (His), lysine (Lys), tryptophan (Trp), arginine (Arg), tyrosine (Tyr), phenylalanine (Phe), and cysteine (Cys), the distance of said amino acids being less than

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three amino acids,
said amino acid sequence being cleavable in the presence of free metal ions,
said amino acid sequence not existing naturally in the protein or peptide to be cleaved; and

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- allowing said protein or peptide to react with the metal ion in a buffer, said buffer further comprising a reducing or oxidizing agent or agents.

2. The method according to claim 1, c h a r a c t e r i z e d in that

the amino acid sequence comprises at least one unit selected from the group comprising $X_1 X_1$, or repeats thereof,

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$X_1 Y_n X_1$, or repeats thereof,

two or more repeats of $X_1 Y_n$,

wherein n is an integer of 1 to 3, and

X_1 is selected from the group comprising His, Cys, Lys and Trp, or

X_1 is His and Y_n is selected from the group comprising Cys, Lys and Trp, or

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X_1 is Cys and Y_n is selected from the group comprising His, Lys and Trp, or

X_1 is Lys and Y_n is selected from the group comprising His, Cys and Trp, or

X_1 is Trp and Y_n is selected from the group comprising His, Cys and Lys, or

wherein n is an integer of 1 to 3, and

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X_1 is selected from the group comprising His, Cys, Lys and Trp, and

Y_n is any amino acid,

or the amino acid sequence comprises the units in different combinations optionally with other amino acids in between.

3. The method according to claim 1 or 2, wherein the length of the amino acid sequence is less than 100, preferably less than 50 amino acids.
- 5 4. The method according to any one of the preceding claims, wherein the length of the amino acid sequence is 2 to 20, preferably 2 to 10 amino acids, more preferably 4 to 8 amino acids.
- 10 5. The method according to any one of the preceding claims, wherein the amino acid sequence comprises two or more repeats of X_1Y_n , wherein $n=1$, X_1 is His and Y_n is any amino acid.
- 15 6. The method according to any one of the preceding claims, wherein the amino acids are selected from the group comprising His, Lys and Trp, more preferably from the group comprising His and Lys, most preferably the amino acid is His.
- 20 7. The method according to any one of the preceding claims, wherein the amino acid is His.
- 20 8. The method according to any one of the preceding claims, wherein the amino acid sequence comprises a sequence selected from the group comprising
- (His)₂, (His)₄ (SEQ ID NO:28), (His)₆ (SEQ ID NO:29), (His)₈ (SEQ ID NO:30) and His-Ser-His-Ala-His-Gly-His-Ala-His-Ser-His-Gly (SEQ ID NO:9).
- 25 9. The method according to any one of the preceding claims, wherein the metal ion is a ion of a metal selected from the group of transition metals, preferably from the group comprising Cu, Co, Ni, Fe, Mn, Cd, Pd, Rh, Ru, Pt, Cr and Zn.
- 30 10. The method according to any one of the preceding claims, wherein the metal ion is a ion of a metal selected from the group comprising Cu, Co, Mn, Cr, Ni, Fe and Zn, preferably from the group comprising Cu and Co.

11. The method according to any one of the preceding claims, wherein the metal ion is a ion of the metal Cu.
12. The method according to any one of the preceding claims, wherein the protein to be
5 cleaved is a recombinant protein.
13. The method according to any one of the preceding claims, wherein the amino acid sequence is constructed at a predetermined cleavage site by genetic engineering methods.
- 10 14. The method according to any one of the preceding claims, wherein the reaction is carried out in the presence of a reagent selected from the group comprising hydrogen peroxide, ascorbate and dithiothreitol or in the presence of a combination of these reagents.
- 15 15. Use of free metal ions selected from the group of Cu^{2+} and Co^{2+} for cleaving a protein or peptide at a predetermined, specific site comprising amino acids selected from the group comprising histidine, lysine, tryptophan, arginine, tyrosine, phenylalanine, and cysteine.